



SEQUENCE LISTING

#4

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<120> COMBINATORIAL POLYKETIDE LIBRARIES
PRODUCED USING A MODULAR PKS GENE CLUSTER AS SCAFFOLD

<130> 30062-20005.02

<140> 09/859,854

<141> 2001-05-16

<150> PCT/US98/08792

<151> 1998-04-30

<150> 60/076,919

<151> 1998-03-05

<150> 08/846,247

<151> 1997-04-30

<150> 08/486,645

<151> 1995-06-07

<150> 08/238,811

<151> 1994-05-06

<160> 44

<170> FastSEQ for Windows Version 4.0

<210> 1

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Module 1 - A BamHI site engineered for the 5'
boundary of the acyltransferase domain.

<400> 1

gcgcagcagg gatccgtctt cgtc

24

<210> 2

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Module 1 - A PstI site engineered for introduction
between the acyltransferase and reductive domains.

<400> 2
 cgcgctctggc tgcagccgaa gccg 24

<210> 3
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Module 1 - A XbaI site engineered for introduction
 at the 3' end of the reductive domain.

<400> 3
 gcgcgggtga gatctaagcc ggcc 24

<210> 4
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Module 2 - A BamHI site engineered for the 5'
 boundary of the acyltransferase domain.

<400> 4
 tccgacgggtg gatccgtggt cgtc 24

<210> 5
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Module 2 - A PstI site engineered for introduction
 between the acyltransferase and reductive domains.

<400> 5
 cggttctggc tgcagccgga ccgc 24

<210> 6
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Module 2 - A XbaI site engineered for introduction
 at the 3' end of the reductive domain.

<400> 6
 gtcggccaga gatctcgaga ggca 24

<210> 7
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Module 3 - A BamHI site engineered for the 5'
 boundary of the acyltransferase domain.

<400> 7
 gacggg'gcgcg gatccgtctt cctg 24

<210> 8
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Module 3 - A PstI site engineered for introduction
 between the acyltransferase and reductive domains.

<400> 8
 cgctactggc tgcagcccgc cgca 24

<210> 9
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Module 3 - A XbaI site engineered for introduction
 at the 3' end of the reductive domain.

<400> 9
 ctcggcaaca gatctgagcg gccca 24

<210> 10
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Module 4 - A BamHI site engineered for the 5'
 boundary of the acyltransferase domain.

<400> 10
 gcgccgcgcg gatccgtcct ggtc 24

<210> 11
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Module 4 - A PstI site engineered for introduction
 between the acyltransferase and reductive domains.

<400> 11
 cgcttctggc tgcagccgca ccgg 24

<210> 12
 <211> 24

<212> DNA
 <213> Artificial Sequence

 <220>
 <223> Module 4 - A XbaI site engineered for introduction
 at the 3' end of the reductive domain.

 <400> 12
 ctcgccaga gatctcaagc cggg 24

 <210> 13
 <211> 24
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Module 5 - A BamHI site engineered for the 5'
 boundary of the acyltransferase domain.

 <400> 13
 actcgccgcg gatccgcgat ggtg 24

 <210> 14
 <211> 24
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Module 5 - A PstI site engineered for introduction
 between the acyltransferase and reductive domains.

 <400> 14
 cggtagctggc tgcagatccc cacc 24

 <210> 15
 <211> 24
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Module 5 - A XbaI site engineered for introduction
 at the 3' end of the reductive domain.

 <400> 15
 gacccgctca gatctcggga ggag 24

 <210> 16
 <211> 24
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Module 6 - A BamHI site engineered for the 5'
 boundary of the acyltransferase domain.

 <400> 16
 tccgccggcg gatccgtttt cgtc 24

<210> 17
 <211> 24
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Module 6 - A PstI site engineered for introduction
 between the acyltransferase and reductive domains.

 <400> 17
 cggtactggc tgcagccgga ggtg 24

 <210> 18
 <211> 24
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Module 6 - A XbaI site engineered for introduction
 at the 3' end of the reductive domain.

 <400> 18
 gacgtggcga gatctccggg ggtg 24

 <210> 19
 <211> 23
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> A PstI site that is in-frame and upstream of XbaI
 in pUC19 that generates this junction at the 5'
 end of the cassette.

 <400> 19
 ctgcaggtcg actctagcct ggt 23

 <210> 20
 <211> 27
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Module rapAT2 (forward) Primer pairs used for PCR
 amplification of rapamycin PKS cassettes.

 <400> 20
 tttagatctg tgttcgtctt cccgggt 27

 <210> 21
 <211> 36
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Module rapAT2 (reverse) Primer pairs used for PCR

amplification of rapamycin PKS cassettes.

<400> 21
tttctgcagc cagtaccgct ggtgctggaa ggcgta 36

<210> 22
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Module rapAT14 (forward) Primer pairs used for PCR
amplification of rapamycin PKS cassettes.

<400> 22
tttgatccg ccttcctggt cgacgggcaa ggc 33

<210> 23
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Module rapAT14 (reverse) Primer pairs used for PCR
amplification of rapamycin PKS cassettes.

<400> 23
tttctgcagc cagtaggact ggtgctggaa cgg 33

<210> 24
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Module rapKR2 (forward) Primer pairs used for PCR
amplification of rapamycin PKS cassettes.

<400> 24
tttctgcagg agggcacgga ccgggcgact gcgggt 36

<210> 25
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Module rapKR2 (reverse) Primer pairs used for PCR
amplification of rapamycin PKS cassettes.

<400> 25
ttttctagaa ccggcggcag cggcccgcg agcaat 36

<210> 26
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
 <223> Module rapDH/KR4 (forward) Primer pairs used for
 PCR amplification of rapamycin PKS cassettes.

<400> 26
 ttctgcagag cgtggaccgg gcggct 26

<210> 27
 <211> 30
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Module rapDH/KR4 (reverse) Primer pairs used for
 PCR amplification of rapamycin PKS cassettes.

<400> 27
 ttttctagag tcaccggtag aggcggccct 30

<210> 28
 <211> 30
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Module rapDH/ER/KR1 (forward) Primer pairs used
 for PCR amplification of rapamycin PKS cassettes.

<400> 28
 tttctgcagg gcgtggaccg ggcggctgcc 30

<210> 29
 <211> 30
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Module rapDH/ER/KR1 (reverse) Primer pairs used
 for PCR amplification of rapamycin PKS cassettes.

<400> 29
 tttctcgagc accacgcccg cagcctcacc 30

<210> 30
 <211> 30
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Module rapDH/ER/KR1 (forward) Primer pairs used
 for PCR amplification of rapamycin PKS cassettes.

<400> 30
 tttctcgagg tcgggtccgga ggtccaggat 30

<210> 31

<211> 30
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Module rapDH/ER/KR1 (reverse) Primer pairs used
 for PCR amplification of rapamycin PKS cassettes.

 <400> 31
 ttttctagaa tcaccggtag aagcagcccg 30

 <210> 32
 <211> 24
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> The junctions at which the PstI and XbaI sites
 were introduced into DEBS.

 <400> 32
 gagccccagc ggtactggct gcag 24

 <210> 33
 <211> 24
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> The junctions at which the PstI and XbaI sites
 were introduced into DEBS.

 <400> 33
 tctagagcgg tgcaggcggc cccg 24

 <210> 34
 <211> 30
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> A primer at which the eryKR6 domain was PCR
 amplified.

 <400> 34
 tttggatccg ttttcgtctt cccaggtcag 30

 <210> 35
 <211> 30
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> A primer at which the eryKR6 domain was PCR
 amplified.

 <400> 35

tttctgcagc cagtaccgct ggggctcgaa

30

<210> 36

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> A primer at which the eryKR6 domain was PCR amplified.

<400> 36

ttttctagag cggtgcaggc ggccccggcg

30

<210> 37

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<223> A primer at which the eryKR6 domain was PCR amplified.

<400> 37

aaaatgcatc tatgaattcc ctccgccca

29

<210> 38

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> The resulting PstI and XbaI junctions engineered into DEBS.

<400> 38

gaacaccagc gcttctggct gcag

24

<210> 39

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> The resulting PstI and XbaI junctions engineered into DEBS.

<400> 39

tctagagacc ggctcgccgg tcgg

24

<210> 40

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Resulting engineered DEBS/rapAT2 junction.

<400> 40
 agtgctccg acggtggatc t 21

<210> 41
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Resulting engineered DEBS/rapAT2 junction.

<400> 41
 ctgcagccgg accgcaccac ccct 24

<210> 42
 <211> 47
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> A synthetic oligonucleotide linker designed to
 allow complete excision of reductive cycle domains
 - designed to generate PstI- and XbaI-compatible
 ends upon hybridization.

<400> 42
 gccggaccgc accacccctc gtgacggaga accggagacg gagagct 47

<210> 43
 <211> 55
 <212> DNA
 <213> Artifical Sequence

<220>
 <223> A synthetic oligonucleotide linker designed to
 allow complete excision of reductive cycle domains
 - designed to generate PstI- and XbaI-compatible
 ends upon hybridization.

<400> 43
 ctagagctct ccgtctccg ttctccgtca cgaggggtgg tgcggtccg ctgca 55

<210> 44
 <211> 12
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> The fusion between the two residues L3455 of DEBS1
 and Q2891 of DEBS3.

<400> 44
 ctactagtc ag 12